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Research report

Sustained medically unexplained physical symptoms in euthymic patients with recurrent depression: Predictive value for recurrence and associations with omega 3- and 6 fatty acids and 5-HTTLPR? ☆

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ABSTRACT

Background: Identification of potentially modifiable risk factors for recurrence in recurrent depression could provide opportunities to improve preventive interventions. In this study we aimed to examine the predictive value of medically unexplained physical symptoms (MUPS) on time to recurrence in recurrent depression. Additionally, to elucidate pathophysiological mechanisms that could explain the relations between MUPS and depression, we investigate the association between a sustained high level of MUPS, and (I) omega (ω)–3 and –6 fatty acid (FA)-status and (II) functional polymorphisms in the promoter region of the serotonin transporter gene (5-HTTLPR).

Methods: Based on three Physical Symptom Checklist (PCS) scores over 12 months, we defined two groups of remitted recurrently depressed patients: 41 patients with a sustained high number of MUPS and 34 patients with a sustained low number or no MUPS. Patients were followed-up for 3.5 years while recurrence of their depression was monitored. In addition, we analyzed patients' erythrocyte's FA-profiles and triallelically genotyped their 5-HTTLPR.

Results: A sustained high level of MUPS predicted consecutive depression recurrence over 3.5 years (adjusted relative risk 2.8). FA-status and distribution of 5-HTTLPR variant frequencies did not differ between patients with sustained high compared to low/absent MUPS-levels.

Limitations: Our sample was relatively small.

Conclusion: Remitted recurrently depressed patients with sustained MUPS have a considerably increased risk of recurrence. Having sustained MUPS is not associated with either erythrocyte ω –3 or –6 FA-levels or 5-HTTLPR polymorphism. Recognition and reducing MUPS in an early state could prevent a (depressive) relapse.

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1. Introduction

Many patients (40–50%) with depression will have more than one episode and therefore suffer from the recurrent

type of this disorder (Mueller et al., 1999; Solomon et al., 2000). Identifying predictors for recurrence in these patients is important for a better understanding of the course of this disease. Well known risk factors for recurrence are: clinical variables (age at onset of first episode, severity of first episode, number of previous episodes, residual symptoms), family history, negative/extreme cognitions, personality (neuroticism), daily hassles, poor social support and maladaptive coping styles (Bockting et al., 2006; Burcusa and Iacono, 2007; Conradi et al., 2008; Fava et al., 2007; ten Doesschate et al., 2010). These predictors explain only part of the variation in

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recurrence. Identification of dynamic, potentially modifiable, risk factors for recurrence in particular could provide an opportunity for developing targeted preventive intervention.

Somatic symptoms, not attributable to a diagnosable medical condition, are common in patients with depression (Demyttenaere et al., 2006; Jain, 2009; Tylee and Gandhi, 2005; Vaccarino et al., 2008). These so-called medically unexplained physical symptoms (MUPS) show a wide variety of severity, ranging from a single, mild and transient symptom to a larger number of more chronic and extremely debilitating ones (Brown, 2007). Insight in the relation between MUPS and depression is interesting because high levels of MUPS in depressed patients may (I) greatly harm their quality of life and increase the burden of depression (Demyttenaere et al., 2006; Munoz et al., 2005), (II) hinder full remission (Karp et al., 2005; Simon et al., 1999; Vieta et al., 2008), and (III) impede treatment response (Greco et al., 2004; Karp et al., 2005). Hence, the need for proper recognition of MUPS and appreciation of their clinical value is unquestionable.

Cross-sectionally, the correlation between depression and MUPS has been well established (Burton et al., 2011; Demyttenaere et al., 2006; Munoz et al., 2005; Tylee and Gandhi, 2005). However, longitudinal studies examining the temporal relationship between depression and MUPS are scarce (Bair et al., 2003). It has been postulated that MUPS, instead of fluctuating in severity parallel to the depressive symptoms, remain present in between depressive episodes (Vaccarino et al., 2009). Therefore, MUPS could be regarded as (I) residual symptoms of a depressive episode, and/or (II) an early indicator of, or a risk factor for a new depressive episode (Judd et al., 1998; Vaccarino et al., 2009). Identifying MUPS as a predictor of recurrence in patients with recurrent depression could be clinically relevant, also because MUPS may represent a dynamic modifiable factor involved in recurrence.

The relation between depression and MUPS may be due to shared underlying biological pathways. In this paper we focus on two of these related pathways. First, the polyunsaturated fatty acid (PUFA)-metabolism, because PUFAs (I) participate in immune regulation (II) determine neuronal membrane stability, and (III) are involved in neurotransmission and signal transduction (Assies et al., 2010; Su, 2009). Depression is associated with lowered omega-3 ($\omega-3$) fatty acid levels and an imbalance between $\omega-3$ and $\omega-6$ PUFAs, which is generally being hypothesized as harmful (Appleton et al., 2008; Assies et al., 2010) and might be linked to somatic manifestations (Su, 2009). Second, another possible underlying mechanism may be serotonergic pathways, because they are considered to play a role in both depression and the development of physical (pain) symptoms (Bair et al., 2003). There is evidence for an association of longer 5-HTTLPR allele mutations with

MUPS, while depression itself is associated either with a longer 5-HTTLPR allele mutation or other 5-HTTLPR mutational variants (Bosker et al., 2010; Hennings et al., 2009; Risch et al., 2009). Lowered omega-3 FA status is related to serotonergic disturbances which offer an etiological pathway for mood and cognitive dysfunction in depression (Su, 2009).

No study thus far has examined the impact of MUPS on the prognosis of recurrence in the recurrent type of depression. Therefore, the first aim of our study was to determine the predictive value of a sustained high level of MUPS for recurrence in euthymic patients with recurrent depression. Second, we aimed to determine whether the biological profiles of the patients with and without a sustained high level of MUPS differ. We hypothesized that a high level of MUPS would be accompanied by an increased $\omega-6/\omega-3$ FA ratio and a higher expressing variant of the 5-HTTLPR polymorphism.

2. Methods

2.1. Study population

The current study was part of the DELTA-study, a randomized clinical trial, investigating the effect of cognitive therapy on recurrence in euthymic patients with ≥ 2 previous major depressive episodes (MDEs) in the last 5 years. Among the exclusion criteria were current or previous mania or hypomania (bipolar disorder), any psychotic disorder (current or previous), alcohol or drug abuse and predominant anxiety disorder. Participants were recruited from psychiatric centers and through media announcement. Recurrence of depression was the main outcome parameter. Since neither type of after-care, nor AD use, was an inclusion or exclusion criterion for the study, with respect to these characteristics, the DELTA sample can be considered representative for patients suffering from recurrent depression. All patients provided informed consent to enter the protocol which was approved by the institutional ethics review committees. The background and methodology of the DELTA-study is described in more detail previously (Bockting et al., 2005).

For our MUPS study two periods of the DELTA-study are of importance (see Fig. 1). The first one, month 12 to month 24 of DELTA, was used to define the MUPS group and includes three moments of assessment: 12 (T_{-12}), 18 (T_{-6}) and 24 (T_0) months after inclusion. The second is the period in which we assessed recurrence. This follow up starts at month 24, defined as T_0 , and runs for 3.5 years.

The DELTA-baseline sample comprised 187 participants of which 15 were excluded because they dropped out of the study immediately after randomization (Bockting et al., 2005). From the remaining 172 patients we included those

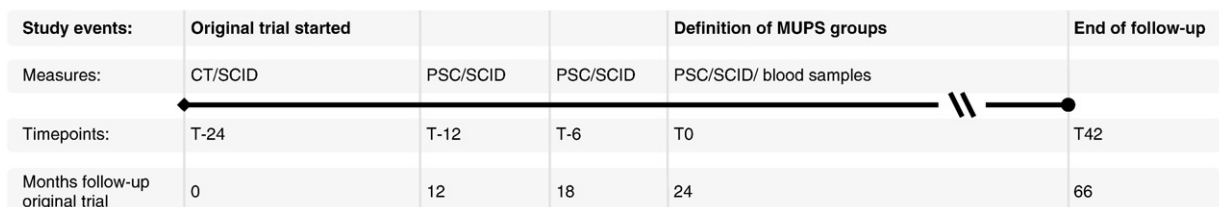


Fig. 1. Time and events schedule.

patients (a) who attended the study-protocol at T_{-12} , T_{-6} and T_0 , (b) had a valid Physical Symptom Checklist (see further) at T_{-12} , T_{-6} and T_0 , (c) who were euthymic (had no DSM IV-R diagnosis of major depressive episode) in the period between T_{-12} and T_0 , and (d) had at least one follow up measurement after T_0 (time at risk > 0). The latter two criteria were necessary requirements for standard Cox regression analysis. Of the 187 patients, 78 patients did not fulfill one or more of the above criteria (24 criterion b, 47 criterion c, and 7 criterion d), resulting in 109 eligible patients.

2.2. Measures

2.2.1. Medically unexplained symptoms

Physical symptoms were assessed three times (T_{-12} , T_{-6} , T_0) using the Physical Symptom Checklist (PSC) (de Waal et al., 2004, 2008). The PSC is a self-report checklist comprising 55 physical symptoms covering most organ systems. Four of these 55 are gender specific (one for men and three for women). These items were excluded to rule out bias by gender. From the 51 remaining items, 10 items measure autonomous symptoms, 11 are general/neurological, 8 musculoskeletal/pain, 13 gastrointestinal, 5 urological/genital items and 4 items are about feeling hot/cold. Each symptom is rated by the patient on a 4-point Likert scale (0–3) with the preceding week as the time frame. For the analyses, each symptom score is dichotomized into 'not present' (scores 0 and 1) or 'present' (i.e. 'bothersome often or most of the time during the previous week', scores 2 and 3). The resulting total symptom score ranges from 0 to 51 and can be considered an indicator of the severity of somatoform disorders and a predictor of health care utilization (Kroenke et al., 1994; Speckens et al., 1996).

At the same time as the PCS assessments, patients were asked in an open interview about their concurrent physical illnesses. Two clinicians (AS and JA), blind to treatment and relapse/recurrence, compared the results from the PSC and the interviews. When a physical illness or complaint mentioned by the patient could explain individual items of the PSC, these items were recoded as 'not present'. The remaining items of the PSC which could not be explained by a physical illness were considered 'medically unexplained physical symptoms'. When doubt remained (i.e. 21 physical complaints), the symptom was regarded as 'explained'.

2.2.2. Definition of MUPS groups

Based on the PSC scores at T_{-12} , T_{-6} and T_0 we defined two patient groups to maximize the contrast: (1) a MUPS+ group comprising 41 patients with a sustained high number of MUPS (i.e. a PSC score ≥ 5 at each of the three assessments), and (2) a MUPS– group, comprising 34 patients with a sustained low number or no MUPS (i.e. a PSC score < 5 at each of the three assessments). This threshold of 5 was based on Escobar's abridged somatization construct SSI 4/6 (de Waal et al., 2008; Escobar et al., 1998). The presence of high levels of symptoms (e.g. ≥ 5) was suggested as a reasonable threshold to designate "cases" in clinical and epidemiological studies. The remaining intermittent group of 34 patients had 5 or more MUPS at some of the 3 assessments and less than 5 MUPS at the other assessments and was excluded from the analyses. In summary, both the MUPS+ and MUPS– groups did not suffer from a depressive episode

during the assessment of the MUPS, and the MUPS that defined that those groups could not be explained by any known physical condition.

2.2.3. Relapse/recurrence

To assess relapse/recurrence, we used the Structured Clinical Interview for DSM-IV (SCID-I) (First et al., 1996) at 3.5 years follow-up (T_{42} : the end of the study). At assessment, current and past depressive episodes were checked by trained SCID evaluators who were blind to treatment condition. Subjects were instructed not to reveal treatment condition to the interviewers (psychologist/research assistants). All interviews were audio taped. Two independent experienced psychiatrists, blind to treatment condition, evaluated all occasions of participants meeting the DSM-IV criteria for MDD. In cases of disagreement, the ratings of the psychiatrists were used. Kappa for inter-rater agreement between the interviewers and psychiatrist on categorization of a relapse/recurrence or no relapse/recurrence was .96, indicating high agreement.

2.2.4. Sample collection of fatty acids

Fatty acids in washed erythrocytes were analyzed at T_0 using capillary gas chromatography, described previously (Assies et al., 2010). Samples were stored at -80°C until analysis.

2.2.5. Genotyping procedures and analysis

For genotyping, two venous blood samples of 10 ml were taken at T_0 , mixed with EDTA to prevent coagulation, and stored at room temperature within 12 h after sampling, until analysis.

Genomic deoxyribonucleic acid (DNA) was isolated using a filter-based method (QIAamp DNA Mini Kit, Qiagen Ltd, United Kingdom). The length of the 5-HTTLPR polymorphism was determined by gel electrophoresis. The region around the polymorphism was amplified by PCR using forward primer *tgtaaaacgacggccagtgcacacctaacccta* and reverse primer *cagggaacagctatgaccaggaggagatcctgggaga* (M13 primer sequence in italic). The PCR reaction was performed in 10 μl containing 1.5 mM MgCl_2 , 0.2 μM forward and reverse primer, 0.1 mM dNTPs, 0.5 Units Hotfire Polymerase (Solis Biodyne, Estonia), Buffer B (Solis Biodyne, Estonia) and 20 ng genomic DNA. The lengths of the four different alleles were: short = 250 bp, long = 298 bp, long + = 320, long ++ = 380 bp. Genotyping of the rs25531 SNP was done by sequencing (Sanger) using Big Dye Terminators (Applied Biosystems). The M13 forward primer *tgtaaaacgacggccagt* was used for sequencing. 10 μl reactions were performed containing 5 ng of a forward primer, 5 μl PCR product, BDT mix (Applied Biosystems) and 2.5 \times BDT buffer (Applied Biosystems). The length of the 5-HTTLPR polymorphism was confirmed by looking at the length of the sequenced PCR product.

2.3. Statistical analysis

Analyses were performed in SPSS statistics 18.0 (SPSS, Inc., 2009, Chicago, IL). The effect of MUPS on recurrence was assessed with Cox regression, which takes into account differences in time at risk and censoring (no recurrence

during the study period). To maximize the contrast we restricted the analyses to the MUPS+ and MUPS– patient groups.

Half of the study sample randomly received 8 sessions of cognitive therapy during the first three months after inclusion in the DELTA study. This therapy prevented recurrence and its preventive effect increased with the number of previous depressive episodes (Bockting et al., 2005). To test whether this intervention modified the relation between MUPS and recurrence we assessed the significance of the 3-way interaction of treatment condition by high MUPS by number of previous episodes' interaction. Because neither the 3-way MUPS by treatment by previous episodes nor the 2-way MUPS by treatment interaction terms were significant, both experimental and control groups were pooled for the Cox-regression analyses. Log–log survival plots showed that the proportional hazard assumption was met.

To examine whether initial differences in relevant variables confounded the effect of sustained MUPS, estimates were adjusted for the potential confounding effects of the following variables by incorporating them as covariates in the Cox regression analyses: gender, marital status, previous depressive episodes, early onset of depression (in years), antidepressant use (yes/no, continuous yes/no; monitored with the Trimbos/IMTA Self Report Questionnaire for Costs Associated With Psychiatric Illness [TIC-P] and assessed information on AD (type and dosage) every 3 months during follow-up), life events (Negative Life Events Questionnaire), Personality Disorder Questionnaire total score (PDQ-4+) and the 90 item Symptom Check List total score (SCL-90) (Derogatis et al., 1973; Hakkaart-van Roijen et al., 2002; Hyler, 1994; Kraaij and De Wilde, 2001).

The triallelic genotypes were reclassified into a biallelic model by their levels of expression. We grouped the genotypes as S'/S' (S/S, L_G/S, L_G/L_G), S'/L_A (S/L_A, L_G/L_A) and L_A/L_A. In the analyses we combined S'/S' and S'/L_A genotypes to compare them with the high-expressing L_A/L_A genotype (Parsey et al., 2006).

Fisher's exact test was used to examine the significance of the association (contingency) between the two kinds of MUPS classification regarding the biallelic model. Independent means t-tests were performed to detect differences in FA concentrations between patients with and without sustained high levels of MUPS.

3. Results

The 75 patients (of the 172) comprising the MUPS groups were comparable to the other 97 patients on gender, educational level, marital status, % Caucasian, AD use, number of previous episodes, and age at onset of first depression, but differed on current age. The MUPS group was on average 3 years older (MUPS groups patients mean age 46.4, sd 8.2; other patients' mean age 43.4, sd 10.2; $t=2.14$ df=170, $p=.033$).

Characteristics of both MUPS groups are summarized in Table 1, which shows that both groups were comparable on the variables tested (all p 's > .10), except there were more women in the MUPS+ group.

Over the consecutive 3.5 year follow-up period (month 24 [T₀] to month 66 [T₄₂] of the original trial) time to recurrence

was significantly longer for patients belonging to the MUPS– group (Fig. 2). In other words, euthymic patients without sustained MUPS stayed significantly longer in remission than patients who suffered from MUPS, a result which holds, both with and without correction for potential confounders. Compared to the MUPS– group, the MUPS+ group had a raw relative risk of 2.6 (Wald(1)=9.38, $p=.002$) and an adjusted relative risk of 2.8 to experience a recurrence.

Physical complaints most frequently mentioned by patients in the MUPS+ group were: pain in the extremities (54% of patients), muscle tension (54%), back pain (51%), shortness of breath, (49%), nausea, (41%) and headaches (37%).

There were no significant differences in total $\omega-3$ and $\omega-6$ PUFAs and total fatty acids between the MUPS+ and MUPS– group (Table 2). The concentrations of C20:4 $\omega-6$ (arachidonic acid; AA), C20:5 $\omega-3$ (eicosapentaenoic acid; EPA), total $\omega-6/\omega-3$, AA/EPA and C20:4 $\omega-6$ /C22:6 $\omega-3$ (AA/DHA) ratios were comparable between both MUPS groups.

Genotype distribution was in Hardy–Weinberg equilibrium in the triallelic ($\chi^2(3)=4.33$, $p=.44$) but not in the biallelic ($\chi^2(1)=4.20$, $p=.04$) model. However, when we stratified the biallelic model into MUPS+ [$\chi^2(1)=3.55$, $p=.06$] and MUPS– [$\chi^2(1)=1.02$, $p=.31$] groups genotype distribution was in Hardy–Weinberg equilibrium in each stratum. Analyses of serotonin transporter gene promoter polymorphisms in recurrently depressed patients with high and low levels of MUPS showed that the 20.0% of the MUPS+ patients and 30.8% of the MUPS– patients had a L_A containing bi-allelic functional profile (L'L' or L'S'). These differences did not reach statistical significance ($\chi^2(1)=.933$, $p=.334$). The groups also did not differ with respect to the triallelic profile (Fisher exact test $p=.831$).

4. Discussion

The aim of this study was, first, to determine whether remitted recurrently depressed patients with medically unexplained physical symptoms (MUPS) have a poor prognosis over a follow up period of 3.5 years, in terms of recurrence of depression. During the follow up period 50% of MUPS– patients and 80% of the MUPS+ patients experienced a new depressive episode. This suggests that a sustained high number of these symptoms over a 12 month period are predictive for subsequent recurrence. This result holds when we adjusted for confounders such as sex and the use of antidepressants, but also when we control for well known illness related predictors for recurrence, i.e. the number of previous episodes, the age of onset of depression and residual symptoms.

The way we defined and assessed MUPS was meant to ascertain as well as possible that these somatic symptoms were not explained by any physical disorder or illness known to the patient. The predictive power of MUPS could also not be explained by residual depressive symptoms as we corrected our analyses for these symptoms. MUPS were also not part of a depressive episode, because patients were free of such episodes when MUPS were assessed. Finally, it is unlikely that the predictive value of MUPS is confounded by antidepressant use, because we corrected our analysis for the use of this medication. One might argue that the reported muscular tension and shortness of breath, may in fact reflect a high level of anxiety. However, the association between MUPS and

the risk of relapse could not be explained by general psychological complaints, including anxiety symptoms. Moreover patients with premorbid anxiety disorders and substance related disorders were excluded in this study. So, the reported MUPS correspond to symptoms that are possibly not related to these often co-morbid disorders.

Somatic complaints represent a risk factor for the subsequent development of depressive symptoms in nonclinical populations (Escobar et al., 2010; Nakao and Yano, 2006; Terre et al., 2003). In clinical populations of depressed patients, physical symptoms, such as pain, are well documented and common (Perugi et al., 2011; Smith, 1992), and may be of greater importance to patients than the mood disturbance (Goldstein et al., 2004). About half of the patients in general practice with depression suffer from a somatoform disorder as well (Perugi et al., 2011). This relationship could be due to anxiety and depression causing (awareness of) physical symptoms, or physical symptoms causing anxiety and depression. Alternatively, this relationship could be explained by a more complex circular relationship (de Waal et al., 2004), and/or the existence of common (genetic) risk factors.

MUPS might theoretically develop into a new depressive episode by two collateral causal pathways. First, simply as an ongoing common pathophysiological process without any intervening components. Second, in our opinion clinically more plausible, by a set of mediating factors. Symptoms of e.g. pain, muscle tension and headache may interfere with access to positive reinforcers (i.e. enjoyable activities), lead to increased feelings of helplessness, and activate negative cognitive schemas or distortions (e.g. catastrophizing) that contribute to depression (Campbell et al., 2003; Vaccarino et al., 2009). Activated depressive cognitions may contribute to a further misinterpretation of physical sensations as being indicative of an underlying illness (Miranda et al., 2002; Lee et al., 2009).

Alternatively, MUPS and depression can be considered as two distinct disorders with a common pathogenetic basis. This basis could be related to the ubiquitously held 'monoamine theory' of depression which holds that depression is related to alterations in key neurotransmitters, including serotonin, dopamine and noradrenalin (Hirschfeld, 2000). These neurotransmitters are also involved in pain modula-

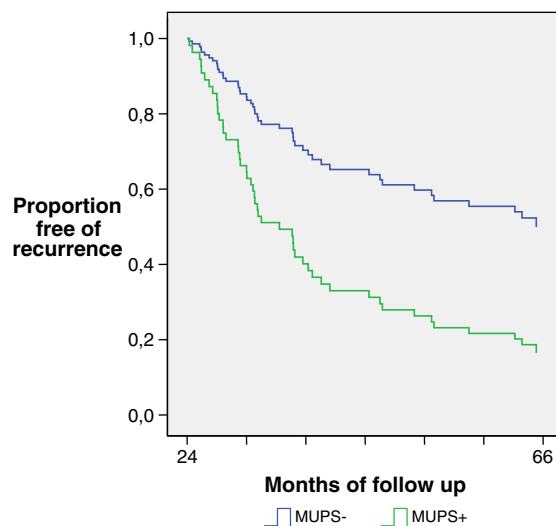


Fig. 2. Recurrence of new depressive episodes in patients with low and high levels of medically unexplained physical symptoms (MUPS).

tion, which under normal conditions functions of and to block or dampen pain signals. Consequently, any 'alterations' in these key neurotransmitters would be expected to affect both pain and depression, and this biological link may help explain the high rates of comorbidity between pain and depression (Bair et al., 2003; Vaccarino et al., 2009).

In the present study, pain in the extremities and muscle tension were the most reported (pain related) symptoms; a finding that is consistent with previous studies frequently reporting muscle soreness in major depressive disorder (Campbell et al., 2003; Fornaro et al., 2011; Kluge et al., 2005; Vaccarino et al., 2009). This symptom might be expected to contribute to the development of, or vulnerability to, other (pain-related) symptoms, including those measured in this study. Although these pain symptoms were accessed as separate categories, they may in fact be mediated by the same underlying factor (Vaccarino et al., 2009). In that case depression and MUPS share common pathways of symptom development. Interestingly, recent developments postulate that depression might be related to mitochondrial dysfunction

Table 1

Characteristics of recurrently depressed patients with high and low levels of medically unexplained physical symptoms (MUPS) at baseline.

Characteristics	Total (n = 75)	MUPS + group (n = 41)	MUPS – group (n = 34)	p value
Female (%)	73	85	59	.009
Age (yr, mean, (SD))	46.4 (8.2)	46.8 (9.0)	45.9 (7.3)	.638
White (%)	98.7	100	97	.325
Single (%)	40	44	35	.456
BMI (kg/m ²) (mean (SD))	26.6 (4.2)	26.4 (4.5)	26.9 (3.7)	.652
Antidepressant medication (%)	59	63	45	.127
Age of first onset (yr, mean (SD))	30.3 (12.7)	29.7 (14.3)	31.0 (10.6)	.643
Previous episodes (median (SD))	5.9 (8.6)	7.1 (11.1)	4.4 (3.4)	.145

All percentages χ^2 test and all continuous variables t tests.

Table 2

Erythrocyte fatty acid concentrations (pmol/10e6 erythrocytes) of recurrently depressed patients with high and low levels of medically unexplained physical symptoms (MUPS).

	MUPS + group (n = 31)	MUPS – group (n = 20)	p value
Eicosapentaenoic acid (C20:5 ω – 3)	3.3 \pm 1.1	3.2 \pm 1.2	.887
Docosahexaenoic acid (C22:6 ω – 3)	14.8 \pm 3.6	14.8 \pm 3.8	.992
Arachidonic acid (C20:4 ω – 6)	70.4 \pm 7.4	70.9 \pm 8.4	.841
C20:4 ω – 6/C20:5 ω – 3 (AA/EPA)	24.3 \pm 8.6	25.6 \pm 11.3	.659
C20:4 ω – 6/C22:6 ω – 3 (AA/DHA)	5.0 \pm 1.1	5.0 \pm 1.2	.984
Total ω – 6/ ω – 3	6.1 \pm 1.2	6.1 \pm 1.2	.940
Total fatty acids	588 \pm 51	579 \pm 59	.619

Independent means t tests.

(Rollins et al., 2009) and that decreased ATP production rates might be present in depressed patients with very high levels of somatic symptoms (Gardner and Boles, 2008).

Somatic symptoms are also thought to be related to higher expressing alleles (L) of 5-HTTLPR, which should facilitate higher reuptake rates of serotonin (Hennings et al., 2009; Narita et al., 2003). However, also links to lower-expressing allele (S) were reported for fibromyalgia (Offenbaeher et al., 1999) and for painful symptoms in major depressive disorder (Smits et al., 2006). We did not find the expected positive association between allelic variation in 5-HTTLPR and a sustained high level of MUPS in patients with recurrent depression. MUPS+ patients with recurrent depression did not differ from MUPS– patients in the frequency of the functional biallelic form of 5-HTTLPR polymorphism. Unfortunately, we examined only a single candidate gene. In regard to the heterogeneity of symptoms in both MUPS and recurrent depression, interactions of multiple genes as biological bases for the psychiatric phenotypes are thought to be more likely. Other explanations for our findings could be that we (I) defined our MUPS groups in terms of longitudinal, repeated symptomatology, whereas other studies used other definitions, and (II) were able to make an absolute contrast with a symptom-free MUPS group. However, the nature of the connection between 5-HTTLPR allelic variants and somatic symptoms remains to be clarified.

Depression is associated with lowered $\omega-3$ fatty acid levels and an imbalance between $\omega-3$ and $\omega-6$ PUFAs. In a previous study (Assies et al., 2010) we showed that in erythrocytes of patients with recurrent depression the concentrations of docosahexaenoic acid and arachidonic acid were lower than in healthy controls, and in addition, patients had a higher $\omega-3$ and $\omega-6$ ratio. In this study, MUPS+ patients with recurrent depression did not differ from MUPS– patients in their $\omega-3$ and $\omega-6$ PUFA status and the ratios ($\omega-6/\omega-3$ AA/EPA, AA/DHA). Riemer et al. (2010) studied the fatty acid status in major depressive patients and patients with comorbid somatoform and depressive disorders. MDD patients and patients with both disorders had significant higher AA/EPA, AA/DHA and total w3/w6 in serum cholesterylesters. However, as in our study, no significant differences for the fatty acid composition of serum phospholipids were detected. We refrain from drawing firm conclusions as factors influencing fatty acid metabolism such as dietary intake of fat and fatty acids, the use of supplements, alcohol consumption, smoking habits and physical activity were not assessed systematically.

Another limitation is the difficulty to distinguish between MUPS and physical symptoms that are part of a broader psychiatric condition, such as depression. Although the selection of appropriate patients for research on MUPS is essential, this judgment may be difficult to make in clinical practice, particularly as the co-morbidity between medically unexplained symptoms, depression and anxiety is extremely high (Smith et al., 2005). Some researchers have tackled this by assuming that a physical symptom cannot be regarded as medically unexplained if it is one of the diagnostic features of major depression or panic disorder (e.g. fatigue, autonomic symptoms etc.) and full diagnostic criteria for that condition are also met (Kroenke, 2003). This is potentially problematic as some physical symptoms can be misdiagnosed as medically unexplained despite only being present during episodes of panic or depression that are not severe enough to meet

formal criteria for these disorders. Conversely, patients often experience physical symptoms of this sort and develop a depressive disorder some time later; many of these patients are understandably reluctant to view their initial physical problems as symptoms of a 'hidden', 'masked' or 'denied' psychiatric illness. In a multicenter, international epidemiologic study, 69% of the patients who met the criteria for depression reported physical symptoms as their only reason for consulting a physician (Simon et al., 1999). However we tried to carefully define participants in terms of the presence of recurrence of depression by undertaking a detailed structured interview and to exclude those with a current relapse from our analyses. Post-hoc, we tested whether the predictive value of MUPS was caused by the fact that some MUPS can be part of depressive symptomatology, and 8 symptoms were removed from the PCS that are (part of the) criteria of depression (DSM-IV), namely; items 1 (feeling tired or having low energy), 2 (easily fatigued without exertion), 8 (sleeplessness), 9 (sleeping a lot), 10 (forgetfulness), 28 (loss of appetite), 29 (weight loss, last month) and 51 (sexual indifference). This did not affect the results.

Considering the various views on MUPS in relation to depression, there are several approaches to and opportunities of integration of different treatments (Fava and Sonino, 2010; Katsamanis et al., 2011). In our study, MUPS were identified as dynamic (potentially modifiable) risk factors for recurrence and could provide the opportunity to preventive intervention. It is mandatory that the somatic symptoms are recognized and reduced in an early state to prevent a (depressive) relapse. In addition, reduction of (pain) symptoms, could establish a better activity level of patients, and might thereby lower the risk of recurrence. Primary treatment of MUPS is also relevant to antidepressant treatment selection, because the mechanisms that may subserve their efficacy for treatment of MUPS are hypothesized to be related to the modulation of both serotonergic and norepinephrinergic neurotransmission. Thus far, for antidepressant treatment of MUPS, there is no clear evidence on the optimum dose, duration of treatment, or long-term outcome. In addition, there exists no firm evidence which antidepressants or other pharmaceutical agent can be regarded as the optimal approach to treat MUPS (Sumathipala, 2007). However, studies suggest that CBT is a promising treatment for MUPS (Jackson et al., 2006; Kroenke, 2007).

5. Conclusion

Sustained medically unexplained physical symptoms predict relapse and recurrence in recurrent depression. This effect seems not to be attributable to shared underlying pathological abnormalities in omega-3 or -6 FA-levels or 5-HTTLPR mutations. More attention for MUPS in patients with recurrent depression could lead to improved (preventive) treatment strategies and outcome measures.

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Conflict of interest

The authors declare that they have no (financial and non-financial) competing interests related to this work. A. Lok had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data-analysis.

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